

SEPARATION AND CHARACTERIZATION OF HUMIC AND FULVIC ACIDS BY GEL ELECTROPHORESIS AND ISOELECTRIC FOCUSING IN POLYACRYLAMIDE GEL*

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I. INTRODUCTION

Humic acid and fulvic acid are natural acidic products with aromatic character and polydisperse properties^[1,2]. In our previous works^[3,4], fulvic acids extracted from two different kinds of weathered coals were fractionated by solvent gradient fractional elution method to obtain a series of fractions with increasing molecular weights. Each fraction was characterized structurally by IR, NMR, ESR, UV and other chemical analyses.

Both humic acid and fulvic acid can be dissociated into macro anions in aqueous solvent systems. Klöcking^[5] and Castagnola^[6] characterized soil humic acid by electrophoresis in polyacrylamide gel. Curvette^[7], Cacco^[8-10] and Thornton^[11] analyzed soil humic acid by resorting to isoelectric focusing (IEF), but their works were far from being conclusive. In our present work, the electrophoretic behaviour of humic acid and fulvic acid from various origins were studied by combining gel electrophoresis with the IEF method and their electrophoretic patterns obtained were correlated with their structural characteristics.

II. RESULTS AND DISCUSSION

The sample preparation and the origin of various humic and fulvic acids used in this work are listed in Table 1. On 20% acrylamide gel concentration, each sample displayed a different electrophoretic pattern (Fig. 1) characterized by several colored zones having somewhat different widths and shades. For a better understanding of the underlying principle of this electrophoretic separation, a fulvic acid (Sample C) and its fractions of known molecular weights were taken and subjected to gel electrophoresis. The results are shown in Fig. 2. It is quite evident that the positions of

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these zones in polyacrylamide gel have a direct bearing on the molecular weights of the samples, while the shades of the zones are related to the amount of the sample applied. The zones denoted by c, d and e in Fig. 2 are attributed to low molecular weight fulvic acid molecules which migrate electrophoretically through the molecular networks of the gel without or with very little restriction, and therefore form discrete zones nearest to the anode. The zones b and b' belong to those molecules having medium molecular weights, the mobilities of which are hindered by the exclusion limit of the gel. The molecules with higher molecular weight did not even enter the network of the 20% gel and remained at the top of the gel as a zone nearest to the cathode. The exclusion limit of the polyacrylamide gel can be varied by changing the concentration of the acrylamide used. The network of the gel became larger as the acrylamide concentration was changed from 20% to 10% and 7%, and the a, b and b' zones, which originally appeared as separated zones in Fig. 1, merged into one single zone as shown in Fig. 3.

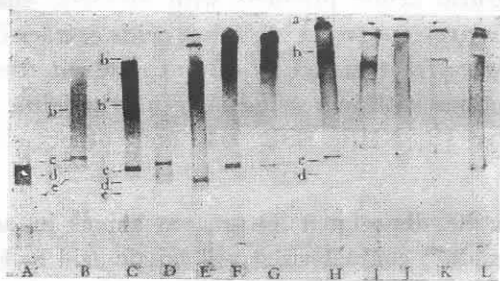


Fig. 1

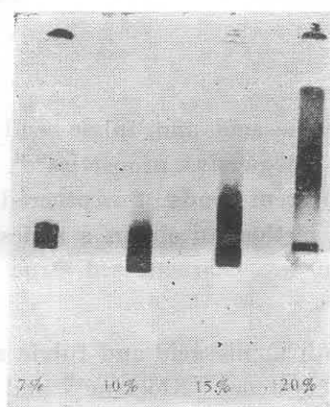


Fig. 3

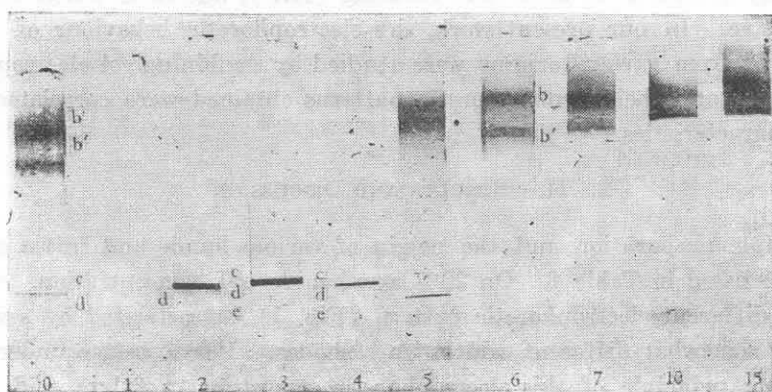


Fig. 2

Humic acid and fulvic acid are not ampholite, and as such they cannot contain positively ionizable functional groups and possess no isoelectric points. In this sense, some controversy still exist as to whether IEF method can be used for the characterization of humic and fulvic acids⁽¹¹⁾. We have found that by using Ampholine of different pH values as carrier ampholite, various humic and fulvic acids were electrically

focused into a number of distinct and discrete bands in polyacrylamide gel within the pH gradient of 2.3—6.5, as demonstrated in Fig. 4.

Table 1

Type and Source of Various Humic Acids and Fulvic Acids

Sample	Type	Source
A	Fulvic acid from weathered coal	Tulufan, Xinjian
B	Fulvic acid from peat	Zhanjian, Guangdong
C	Fulvic acid from weathered coal	Gongxian, Henan
D	Fulvic acid from weathered coal	Datong, Shanxi
E	Humic acid from weathered coal	Manzhouli, Heilongjiang
F	Humic acid from weathered coal	Tulufan, Xinjiang
G	Humic acid from peat	Lianjiang, Guangdong
H	Humic acid from chernozem soil	Gannan, Heilongjiang
I	Humic acid, practical	K & K Lab. Inc., New York
J	Fulvic acid from river sediment	
K	Fulvic acid from river water	
L	Fulvic acid from sea water	Bohai Gulf, Shandong
M	Salicylic acid + gallic acid	

This kind of electric focusing was also observed for organic acids, e.g. salicylic acid and gallic acid which were focused in the gel at pH values corresponding to the dissociation constants (pK_a) of the acids (3.14 for salicylic acid and 4.41 for gallic acid) as shown in Fig. 4M. Humic acid and fulvic acid are constituted of various fused

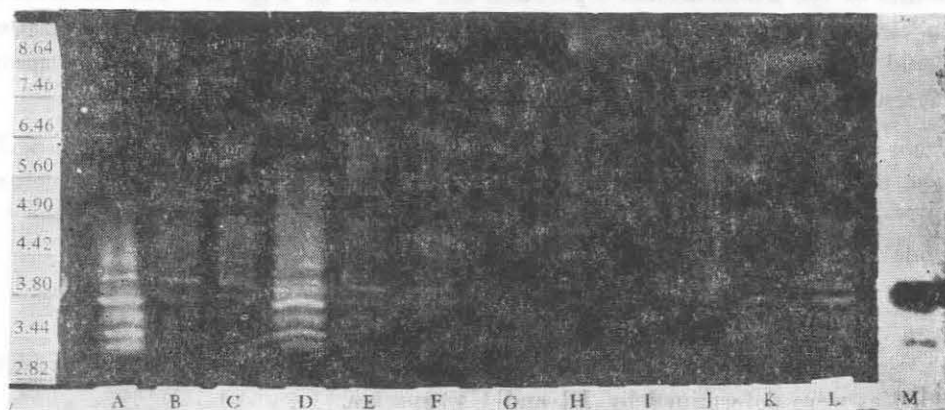


Fig. 4

ring compounds with carboxyl as the main functional group, which exhibit different pK_a values due to the differences in chemical environment. Therefore the components of humic and fulvic acids as well as other organic acids can be separated from each other by IEF. From the IEF patterns of various fractions of sample C (Fig. 5), it can be seen that each fraction which is more or less homogeneous in molecular weight than the original fulvic acid, can further be separated into several bands indicating the heterogeneity in the composition of the fractions. For lower molecular weight fractions there were more bands in the lower pH range, while higher molecular weight

fractions had more bands in the higher pH range. This separation phenomenon is in good accord with our previous experimental results in that the lower molecular weight fractions contain more carboxyl groups than the higher molecular weight ones do.

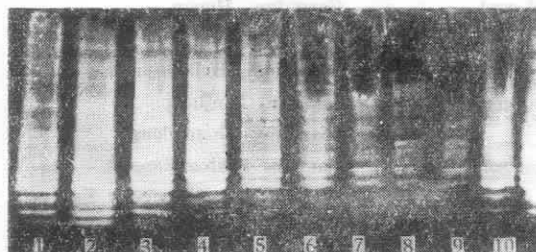


Fig. 5

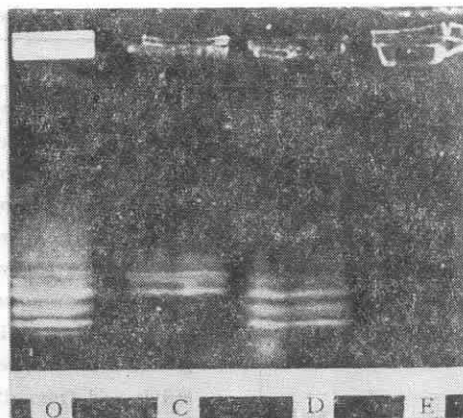


Fig. 6

Gel electrophoresis and IEF belong to two different separation mechanisms, hence the two-dimensional procedure using gel electrophoresis in the first dimension and IEF in the second, was adopted in our present work. After separation of fulvic acid by electrophoresis in a slab of polyacrylamide, the sharp colored zones c, d and e were sectioned and embedded into another slab of polyacrylamide gel, and then subjected to IEF. The results of the 2-dimensional separation are shown in Fig. 6. The electrophoretic pattern clearly demonstrated the value of this procedure for the characterization of humic and fulvic acids. Each dimension separates humic acid or fulvic acid according to different parameters for ready reference or comparison.

From the above experimental results, it is evident that humic and fulvic acids are composed of mixtures of complex molecules having rather similar chemical structure but with different molecular weights. Attempts are now being made to isolate the individual zone or band from the gel for structural studies.

III. MATERIALS AND METHODS

Fulvic acids and humic acids. All samples were used as received. Their identities and purities were determined by IR and UV spectra.

Gel electrophoresis. The gel electrophoresis was carried out in the conventional way^[12].

Isoelectric focusing. IEF was performed with a LKB 217 Multiphor apparatus according to the procedure outlined in the operation manual.

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